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Fabrication of microfluidic devices using dry film photoresist for microchip capillary electrophoresis

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Abstract

An inexpensive, disposable microfluidic device was fabricated from a dry film photoresist using a combination of photolithographic and hot roll lamination techniques. A microfluidic flow pattern was prefabricated in a dry film photoresist tape using traditional photolithographic methods. This tape became bonded to a poly(methyl methacrylate) (PMMA) sheet with prepouched holes when passed through a hot roll laminator. A copper working electrode and platinum decoupler was readily incorporated within this microchip. The integrated microchip device was then fixed in a laboratory-built Plexiglas holder prior to its use in microchip capillary electrophoresis. The performance of this device with amperometric detection for the separation of dopamine and catechol was examined. The separation was complete within 50 s at an applied potential of 200 V/cm. The relative standard deviations (RSD) of analyte migration times were less than 0.71%, and the theoretical plate numbers for dopamine and catechol were 3.2×10^4 and 4.1×10^4, respectively, based on a 65 mm separation channel.

Keywords: Microchip capillary electrophoresis; Amperometric detection; Dry film photoresist; Microfluidic devices

1. Introduction

Miniaturized microfluidic devices in which features of photolithographic technologies and capillary electrophoresis (CE) are combined, have recently become a major focus of interest for the preparation of micro total analysis systems (μTAS, also known as lab-on-a-chip systems). Microfluidic devices have been used in electrophoretic separations of a variety of biochemical and chemical analytes [1–4]. These devices have typically been prepared from glass [5–9], quartz, silicon, and a range of polymeric materials [10–16]. Polymeric materials, such as poly(methyl methacrylate) (PMMA) [10–13] and poly(dimethylsiloxane) (PDMS) [14–16], have great potential for the large-scale fabrication of disposable microfluidic device for use in analytical systems, due to their ease of fabrication, low cost, and great versatility. For polymeric devices, a number of processing techniques can be used to create the required network of microchannels, and all of these approaches have been employed and examined for use in the fabrication of polymeric microfluidic devices including photolithography, wire imprinting, hot embossing, powder ablation, laser photoablation, casting, and injection molding [17–23].

In recent years, disposable plastic-based substrates have become extremely popular for use in microfluidic applications owing to the fact that they are easily and inexpensively fabricated. Sudarsan and Ugaz demonstrated the use of printed circuit technology for the fabrication of plastic-based microfluidic devices [24]. do Lago et al. established a dry process for the production of microfluidic devices based on a xerographic process and the lamination of laser-printed polyester films [25].

Although most photoresists are generally considered to be sacrificial materials, liquid-type negative photoresists, such as SU-8, has been used to create microchannels within microfluidic chips [26–28], and these may play an important role as a structural component of a microfluidic device. Concerning the use of a photoresist as a structural material, the thickness of the photoresist determines the depth of the microchannel; therefore, controlling over the thickness of the photoresist is extremely important in this process. Alternatively, the dry film photoresist was originally developed for printed circuit board (PCB) fabrication could be used. Compared to a liquid photoresist, the dry film photoresist offers a variety of advantages, including good conformability, excellent adhesion to other substrates, uniform distribution, no liquid handling, low exposure energy, and short

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processing time [29]. Furthermore, a commercially available dry film photoresist itself has a uniform thickness, since it is used for constructing micro channels with a specified depth.

In the present study, we report on the fabrication and application of a new plastic microchip that features a negative dry film photoresist (an acrylate-based photopolymer) as a structural material laminated in a PMMA sheet. Off-channel amperometric detection was employed to evaluate the performance of the dry film photoresist-based microchip. A platinum wire electrode serving as a decoupler along with a copper working electrode, which was incorporated into the bottom PDMS sheet. Catechol was used to demonstrate the performance of the microchip CE with an amperometric detector. Dopamine was added to the catechol to evaluate the efficiency of separation of the microchip CE.

2. Experimental

2.1. Chemicals

The 75-μm negative dry film photoresist (AF-5075) was obtained from CCP (Hsinchu, Taiwan). PMMA sheets were purchased from a local hardware store. The Sylgard 184 prepolymer and its curing agent were purchased from Dow Corning (Midland, MI, USA). Disodium hydrogenphosphate, sodium dihydrogenphosphate, and other electrophoresis chemicals were obtained from Fluka (Buchs, Switzerland). Catechol and dopamine were purchased from Sigma (St. Louis, MO, USA). All chemicals were of analytical reagent grade; stock solutions were prepared before each experiment and were stored under refrigeration in the dark. Water was purified with a Milli-Q water system (Millipore, Bedford, MA, USA) and solutions were filtered through 0.45-μm MFS-13 filters (Advantec, Dublin, CA, USA).

2.2. Apparatus

The photolithographic procedures involved the use of a Tah-Hsin TCC-6000 hot roller (Taipei, Taiwan) for pressing, a Union EMA-400 UV aligner (Tokyo, Japan) for exposing, and an auto-development machine for developing. The detection system was a CH 8021b electrochemical analyzer (Austin, TX, USA) coupled to the working, auxiliary, and reference electrodes through sockets. The working electrode was a 50 μm 99.99% copper wire and was given as a gift from Yeou-Chuen Wire (Taoyuan, Taiwan). A Major Science MP-5000-250P high-voltage power supply system (Taipei, Taiwan) with adjustable voltage ranging from 0 to +5 kV was used to power the microchip CE separation.

2.3. Microchip fabrication and assembly

The complete procedure used in the chip fabrication is illustrated in Fig. 1. The photolithographic masks were designed using standard computer software (AutoCAD 2000) and transferred to a transparent film. The microchannel on the mask was represented by a 100-μm-wide black line. The PMMA substrate had a size of 30 mm × 85 mm and a thickness of 1 mm. The

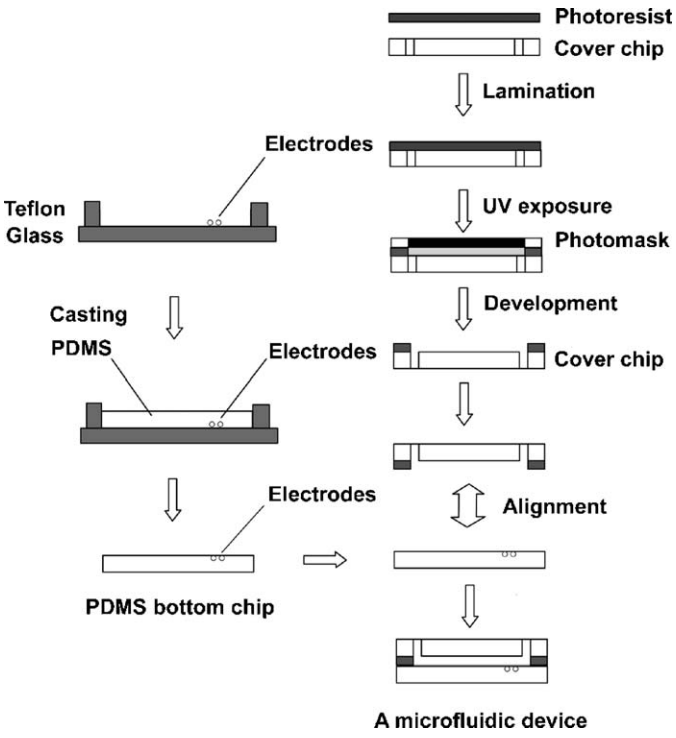
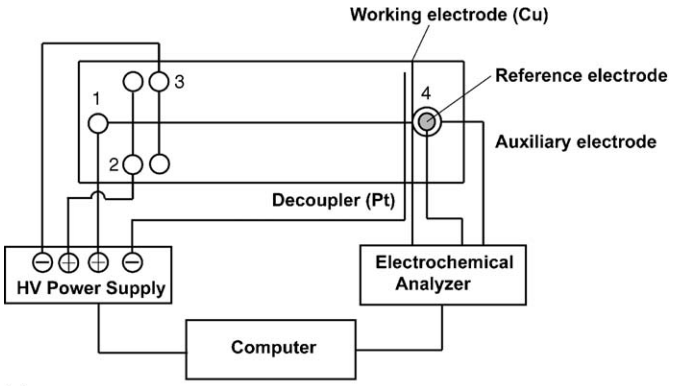


Fig. 1. Schematic diagram of the dry film photolithographic process and lamination procedures for integrating the microchip with an electrochemical detector. The Pt wire decoupler and Cu wire working electrode were embedded in the bottom PDMS sheet.

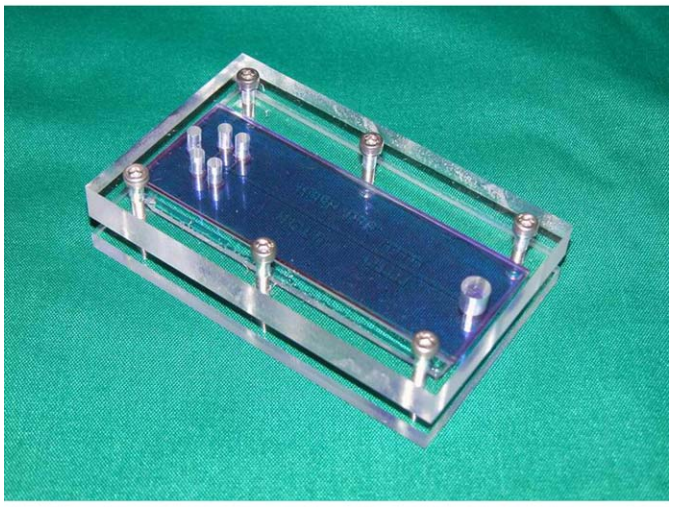
PMMA substrate was washed with water and ethanol and then dried under a flow of high-pressure air from a compressor. A dry film photolithography fabrication technique was used to create the microfluidic channels in the PMMA-based chip in a yellow-light environment. In a typical run, the PMMA substrate was hot rolled on a 75-μm-thick layer of negative dry film photoresist (30 mm × 85 mm) before being covered with the photomask and exposure to UV light. According to the properties of this negative photoresist, the parts which were covered by the pattern of the mask were rinsed out upon development with a 1% aqueous sodium carbonate solution. The developing conditions were controlled by an automated developing machine. To strengthen the structure of the dry film photoresist, the microchip was subjected to a post bake under a 400-W UV light source for 5 min after its production.

Electrodes (Pt and Cu wires) were placed 2 mm apart on a laboratory-built Plexiglas mold with two fillisters to fix the electrodes. Before placing electrodes on the mold, they were cleaned with ethanol. A mixture of Sylgard 184 prepolymer and curing agent (10:1) was stirred thoroughly and degassed in a vacuum for 15 min. The prepolymer mixture was then poured onto the Plexiglas mold and cured at 60 °C for 1 h to form a 2-mm thick PDMS sheet (30 mm × 85 mm).

Fig. 2a shows a schematic illustration of a laboratory-built microchip CE with the amperometric detection system used in this study. The effective separation length of microchannel was 65 mm from the injection zone to the working electrode with a double-T injection channel design. As shown in Fig. 2b, the



(a)



(b)

Fig. 2. Schematic diagram and photographs of the microchip CE system. (a) Layout of the microchip CE with electrochemical detection. (b) Photograph of an electrophoresis microchip fixed in a laboratory-built Plexiglas chip holder.

microchip assembly with electrodes was fixed in a laboratory-build Plexiglas chip holder. Six stainless steel screws were used to house the microchip. The cover holder also serves as sample, buffer, and electrode reservoirs with five 3-mm holes and one 6-mm holes. To prevent leakage of the solution, the dry-film-based chip was clipped between two pieces of PDMS; one piece contained the electrode and the other contained six holes corresponding to the reservoirs on the dry-film-based chip. Because of the elasticity of PDMS, when the holder was compressed tightly, the reservoirs were sealed in a manner analogous to the operation of an O-ring. No leakage from the reservoirs or channels was observed. The electrodes were positioned beneath the separation channel in order to allow buffer to flow past the detector. The copper wire functioned as the working electrode and was located 1 mm before the channel outlet. The Pt wire served as the ground electrode as well as the decoupler.

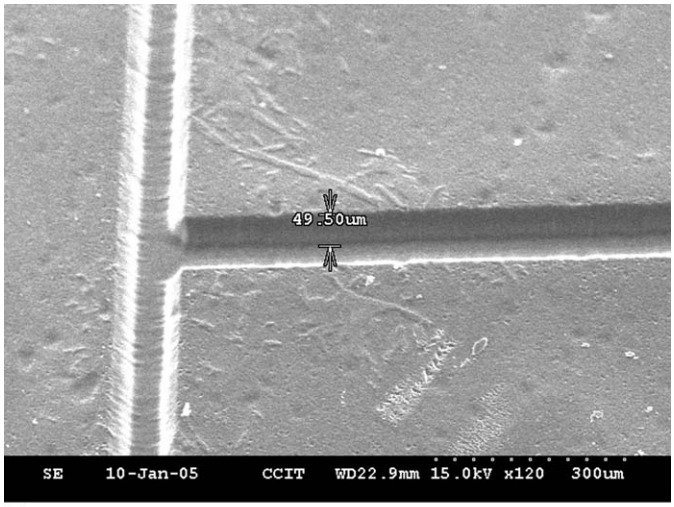
2.4. Procedure

Before conducting each electrophoresis experiment, the channels of the microchip were rinsed with purified water and 1 mM phosphate buffer (pH 7.4) for 10 min each. All standard solutions of analytes (1 mM) were prepared in water, stock solutions were diluted with running buffer to the desired concentrations. The reservoirs were filled with running buffer and the sample and a sample injection potential of 100 V/cm was then applied for 15 s. Separation was then initiated by switching to different voltages across the separation channel.

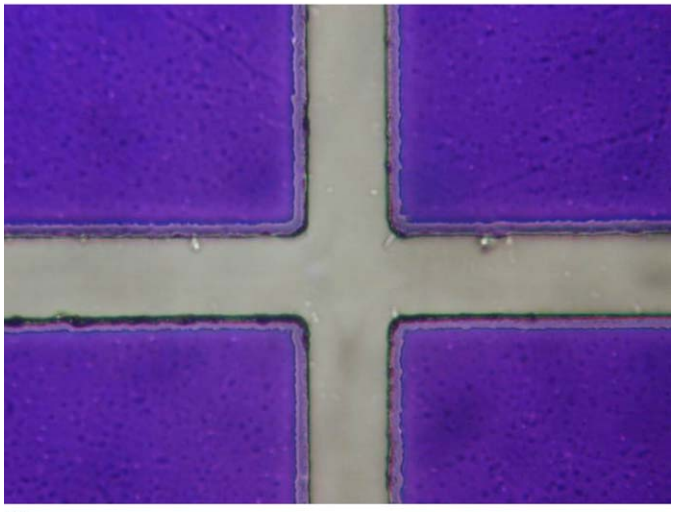
3. Results and discussion

3.1. Fabrication of microchip using dry film photoresist

Prior to the assembly of the microchip, the quality of the microchannels was evaluated by optical microscopy. Fig. 3 shows an SEM image and a photograph of the micro channels on the substrate, produced by dry film photoresist and conventional photolithographic techniques. The observed sidewall



(a)



(b)

Fig. 3. Microscopic images of channels in microchips: (a) SEM image showing a microchannel fabricated in a dry film photoresist and (b) photographic image showing a cross section of microchannels.

images, depicting smooth surfaces and sharp edges, reveal that the microchannel fabricated using dry film photoresist was adequate.

This dry film photolithographic method is a mature and readily accessible technique that can be performed simply and continuously without the need for a restrictive and expensive clean room operating environment. This process can be performed without the use of a spin coating apparatus and does not include time-consuming baking procedures. It is obviously a rapid alternative to the use of a SU-8 liquid type photoresist. Thus, this dry film photolithographic technique could be used for the rapid integration of prototype microchips.

3.2. Performance of microchip CE

After conditioning the microchip in the microchip CE system by running buffer, it was used in the measurement of 500 μM catechol, in order to evaluate the performance of the device. The catechol migrated within 50 s under a 200 V/cm electrical field. The peak width at half height was less than 0.60 s, superior to the same analyte detected using an end-channel detector [30,31]. The sharp analyte peak is probably due to the off-channel detection which eliminates the band-broadening effect [32,33].

To evaluate the performance of the microchip CE device, 50 runs of injection and separation sequences were conducted on 500 μM catechol. Some selected results are presented in Fig. 4. Using a pH 7.4 buffer and 200 V/cm field strength, the average migration time for catechol was 45.8 s. The relative standard deviation (RSD) of the migration time was 1.01% ($n = 5$). We also investigated chip-to-chip reproducibility by running three CE microchips. Each chip led to similar RSD for the migration times. Because migration time is dependent on the distance between the injection zone and the working electrode, the absolute value for migration time should be calibrated for each different microchip.

The calibration curve for catechol was linear from 10 to 1000 μM with a correlation coefficiency (r) that exceeded 0.9996. As calculating from the 10 μM catechol signal, the limit of detection was 730 nM (S/N = 3). These results indicate that the amperometric detector in the microchip displayed a well-defined concentration dependence.

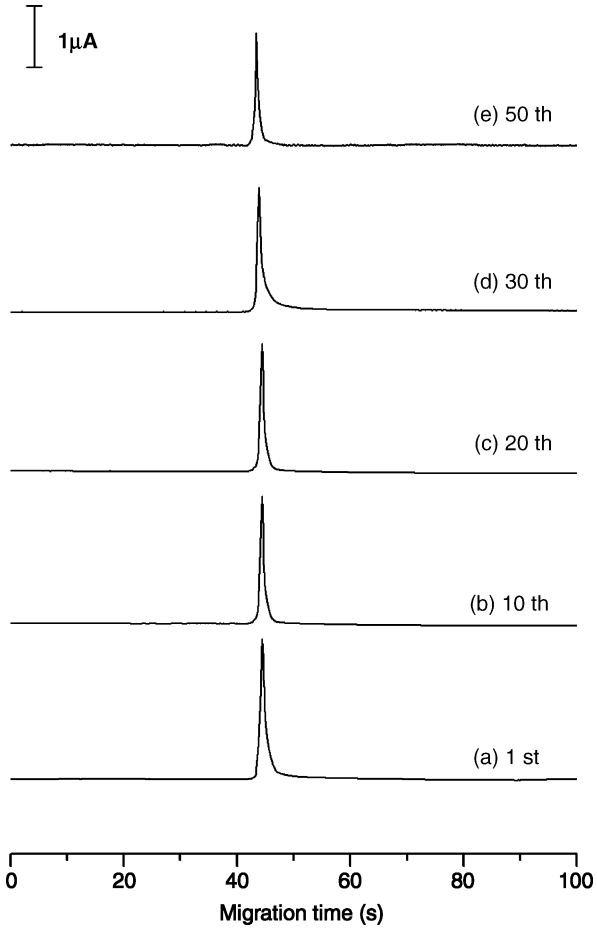


Fig. 4. Five electropherograms of catechol from a total of 50 consecutive runs, showing the first on the bottom and the 50th on the top. Conditions: sample injection: at 150 V/cm for 15 s; separation channel: 65 mm; separation voltage: 200 V/cm; running buffer: phosphate buffer (1 mM, pH 7.4); analyte: 500 μM catechol. Detection voltage of working electrode is +0.3 V (vs. Ag/AgCl).

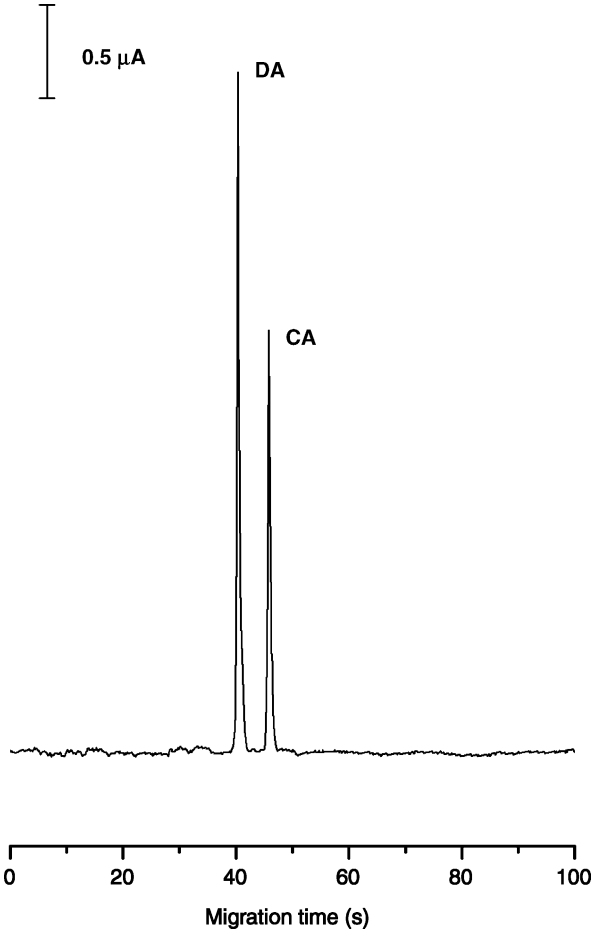


Fig. 5. Electropherogram from the separation of a mixture of dopamine (DA) and catechol (CA). Conditions: sample injection: at 150 V/cm for 15 s; separation channel: 65 mm; separation voltage: 200 V/cm; running buffer: phosphate buffer (1 mM, pH 7.4); analytes concentration: 500 μM. Detection voltage of working electrode is +0.3 V (vs. Ag/AgCl).

3.3. Separation of dopamine and catechol by microchip CE

The separation performance of the microchip CE device was subsequently examined by the separation of dopamine and catechol with the amperometric detector. Fig. 5 shows an electropherogram for the separation of dopamine and catechol by microchip CE. An adequate separation was achieved within 50 s. The average migration times of dopamine and catechol were 30.3 s and 45.8 s ($n = 5$), respectively. The RSD for analyte migration time was less than 0.71% and the RSD for analyte peak height was less than 3.0%. The peak widths at half height were less than 1.0 s for both analytes. The resolution between the two analytes was 2.87. The theoretical plate numbers (N) for dopamine and catechol were 3.2×10^4 and 4.1×10^4 based on a 65 mm separation channel, respectively. The results of the present study clearly demonstrate that this dry film photoresist based microchip can be used successfully in a microchip CE system. Finally, the simple fabrication process of the dry film photoresist-based microchip permits the rapid and convenient replacement of microchips in microchip CE platforms.

4. Conclusions

The dry film photolithographic technique is an alternative and effective method for the manufacture of plastic microchips. The advantages of using this dry film photoresist-based microchip in microchip CE systems include rapid fabrication, low cost, and ease of fit into the chip holders. Thus, other new concepts in the design and fabrication of μTAS microchip can be exploited.

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